Carbapenem-Resistant Pseudomonas aeruginosa–Carrying VIM-2 Metallo-β-Lactamase Determinants, Croatia

To the Editor: Carbapenem-hydrolyzing enzymes of the VIM-type (six different variants are known: VIM-1, VIM-2, VIM-3, VIM-4, VIM-5, and VIM-6) are new molecular class B metallo-β-lactamases. These enzymes have recently been identified in carbapenem-resistant isolates of Pseudomonas aeruginosa and other gram-negative nonfermenters from European countries in the Mediterranean basin (Italy, France, Greece, Spain, Portugal, and Turkey), as well as in Far East countries (Korea, Taiwan, and Singapore) and the United States (1–3, Midilli et al., GenBank accession no. AY144612, Koh et al., GenBank accession no. AY165025). Similar to blaIMP, blaVIM genes are located on mobile gene cassettes inserted in the variable regions of integrons (1), a condition that provides a wide potential for expression and dissemination in gram-negative pathogens. VIM enzymes possess the broadest range of substrate hydrolysis and can degrade virtually all β-lactams, except monobactams (4).

According to a recent report, the overall resistance rate to imipenem in P. aeruginosa isolated from 17 representative laboratories in Croatia was 11% (range 0%–20%) (5). However, molecular basis of carbapenem resistance was not investigated.

In October 2000, two P. aeruginosa isolates with an unusual resistance profile were isolated from two Croatian patients (66 and 74 years of age, respectively) who underwent hysterectomies at the Split University Hospital. Both isolates were cultured from urine a week after surgery; a urinary catheter had been used for both patients who had become febrile and had signs and symptoms of urinary tract infection. Analysis of the macrorestriction profiles of chromosomal DNA of the two isolates by pulsed-field gel electrophoresis, carried out as described previously (6), indicated that the two isolates were clonally related (the two profiles were apparently identical). In routine antibiotic susceptibility testing, done by disk diffusion, both isolates showed a multidrug-resistant phenotype, including ureidopenicillins, piperacillin, piperacillin-tazobactam, ceftazidime, cefoperazone, cefepime, aztreonam, ciprofloxacin, gentamicin, netilmicin, imipenem, and meropenem. MICs to imipenem and meropenem were high (>128 μg/mL). These findings suggested production of an acquired carbapenemase. In fact, crude extracts of the two isolates exhibited carbapenemase activity in a spectrophotometric assay (7) (imipenem hydrolyzing–specific activity was, in either case, >170 nmol/min/mg protein).

A colony blot hybridization, carried out as described with a blaIMP and a blaVIM probe (6), yielded a positive result with the latter probe. Polymerase chain reaction (PCR) amplification of the variable region of class 1 integrons, carried out as described previously by using primers designed on the 5′- and 3′-conserved segments of the integron (8), yielded a 4-kb amplification product from either isolate. Direct sequencing of these amplification products showed, in both cases, the presence of a blaVIM_2 allele located in a gene cassette inserted in the attI site of a class 1 integron.

The metallo-β-lactamase determinant was not transferred to Escherichia coli MKD135 or P. aeruginosa 10145/3 (9) in diparental mating experiments conducted on solid medium (the sensitivity of the assay was ≥1x10^4 transconjugants per donor). Plasmid extraction was performed with several techniques, including lysis with sodium dodecyl sulfate (10) and alkaline lysis conducted with a conventional method (10) or with the Nucleobond BAC100 system (Macherey-Nagel, Duren, Germany). Extraction of whole genomic DNA was also performed, as described (8). Plasmid DNA was not detected in any of these preparations, either when analyzed by agarose gel electrophoresis or after Southern blot hybridization analysis with a blaVIM probe (6), yielding a positive result with the latter probe.

Polymerase chain reaction (PCR) amplification of the variable region of class 1 integrons, carried out as described previously by using primers designed on the 5′- and 3′-conserved segments of the integron (8), yielded a 4-kb amplification product from either isolate. Direct sequencing of these amplification products showed, in both cases, the presence of a blaVIM_2 allele located in a gene cassette inserted in the attI site of a class 1 integron.

This work was supported by the European research network on metallo-β-lactamases within the TMR program (contract no. FMRX-CT98-0232) and by grant “M.I.U.R” (no. 2001068755_003).

Sanda Sardelic,* Lucia Pallecchi,† Volga Punda-Polic,*, and Gian Maria Rossolini†

*University Hospital and School of Medicine, Split, Split, Croatia; and †University of Siena, Siena, Italy

References

Rickettsia felis in the United Kingdom

To the Editor: Rickettsia felis is a bacterium transmitted by the cat flea (Ctenocephalides felis), which also acts as a reservoir by means of transovarial transmission (1–3). The distribution of R. felis is potentially as wide as that of its insect host, and to date, its presence has been confirmed in cat flea populations in North and South America and southern Europe (4,5). R. felis was first identified as a human pathogen in 1994 (6), and cases of “flea-borne spotted fever,” which have signs and symptoms of febrile illness, have now been reported. PCR-positive fleas were collected from 4 dogs and 14 cats from Bristol, Dorset, and Northern Ireland. Taking into account the number of fleas in each pool, we estimate that 6% to 12% of the fleas collected were infected with R. felis.

This study represents the first description of a spotted fever group rickettsia endemic to the United Kingdom. The species detected, R. felis, has clear public health implications. The bacterium appears to be widely distributed within the country, infecting a geographically dispersed population of Ct. felis. Up to 12% of Ct. felis may be infected with R. felis, a flea that is by far the most common species of ectoparasite encountered on cats and dogs in the U.K.